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Mucosal Macrophages in Intestinal Homeostasis and Inflammation

Allan McI Mowat · Calum C. Bain

Institute of Infection, Immunology and Inflammation, University of Glasgow, Glasgow, UK

Key Words

Macrophage · Intestine · Inflammation · Homeostasis

Abstract

Intestinal macrophages are essential for local homeostasis and in keeping a balance between commensal microbiota and the host. However, they also play essential roles in inflammation and protective immunity, when they change from peaceful regulators to powerful aggressors. As a result, activated macrophages are important targets for treatment of inflammatory bowel diseases such as Crohn's disease. Until recently, the complexity and heterogeneity of intestinal macrophages have been underestimated and here we review current evidence that there are distinct populations of resident and inflammatory macrophages in the intestine. We describe the mechanisms that ensure macrophages remain partially inert in the healthy gut and cannot promote inflammation despite constant exposure to bacteria and other stimuli. This may be because the local environment 'conditions' macrophage precursors to become unresponsive after they arrive in the gut. Nevertheless, this permits some active, physiological functions to persist. A new population of pro-inflammatory macrophages appears in inflammation and we review the evidence that this involves recruitment of a distinct population of fully responsive monocytes, rather than alterations in the existing cells. A constant balance be-

tween these resident and inflammatory macrophages is critical for maintaining the status quo in healthy gut and ensuring protective immunity when required.

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Introduction

Macrophages (m Φ) are one of the most abundant leucocytes in the intestine of all mammalian species and this is probably the largest population of mononuclear phagocytes in the body [1]. M Φ are found throughout the intestinal tract of all mammals, both in the mucosa and deeper layers such as the submucosa and muscularis mucosae. However, they are found in greatest numbers in the lamina propria which forms the core of the mucosa underlying the surface epithelium (fig. 1). They are rare in the epithelium itself, although some specialised populations of mononuclear leucocytes may be able to send processes through the epithelium from the lamina propria to sample luminal contents (see below). Although few detailed studies have been conducted, mucosal m Φ numbers in different parts of the intestine appear to correlate closely with the relative bacterial load and thus are highest in the colon, where the number of commensal bacteria is in excess of 10^{12} organisms/ml [2]. Their numbers are also decreased in the intestine of germ-free mice [3]. Interest-

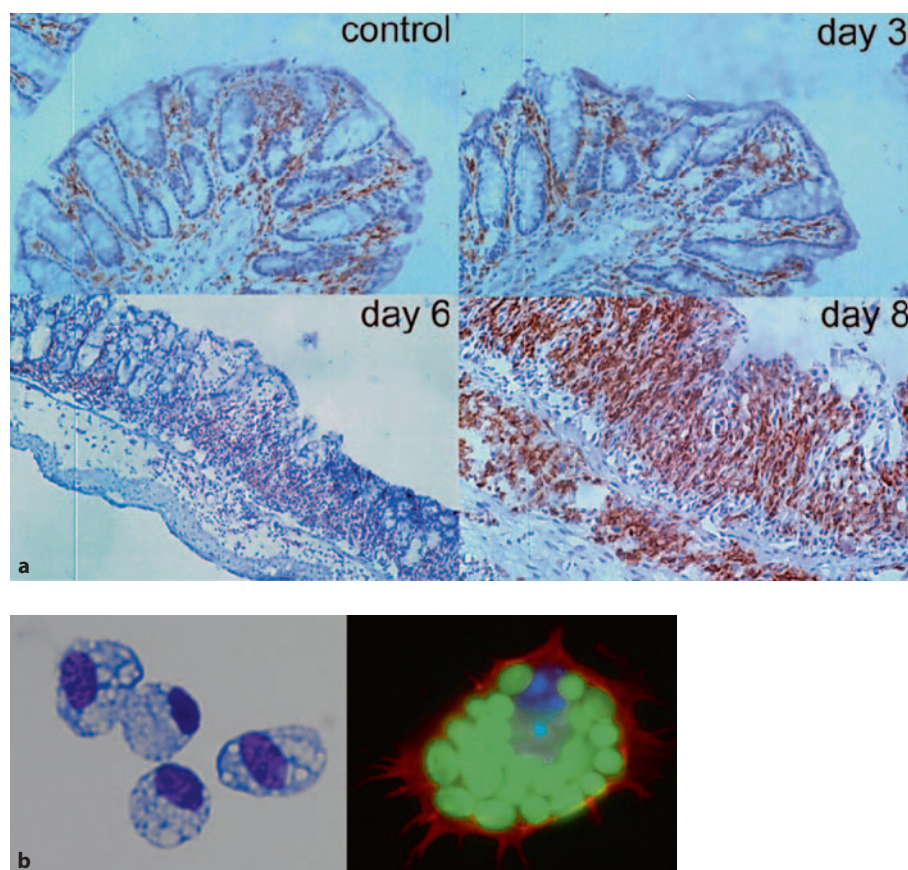


Fig. 1. Location and morphology of mΦ in healthy colon. **a** F4/80+ cells in control mouse colon and 3, 6 and 8 days after induction of DSS colitis, showing the presence of mΦ in healthy intestine and the intense infiltrate in inflammation. Reproduced with permission from Stevceva et al. [109]. **b** Purified F4/80+ class II MHC+ mΦ from normal mouse colon show the morphological appearances of activation, with abundant foamy cytoplasmic granules and they phagocytose FITC-labelled zymosan particles.

ingly however, mΦ are also frequent in more proximal regions of the intestine where bacteria are rare, such as the small intestine.

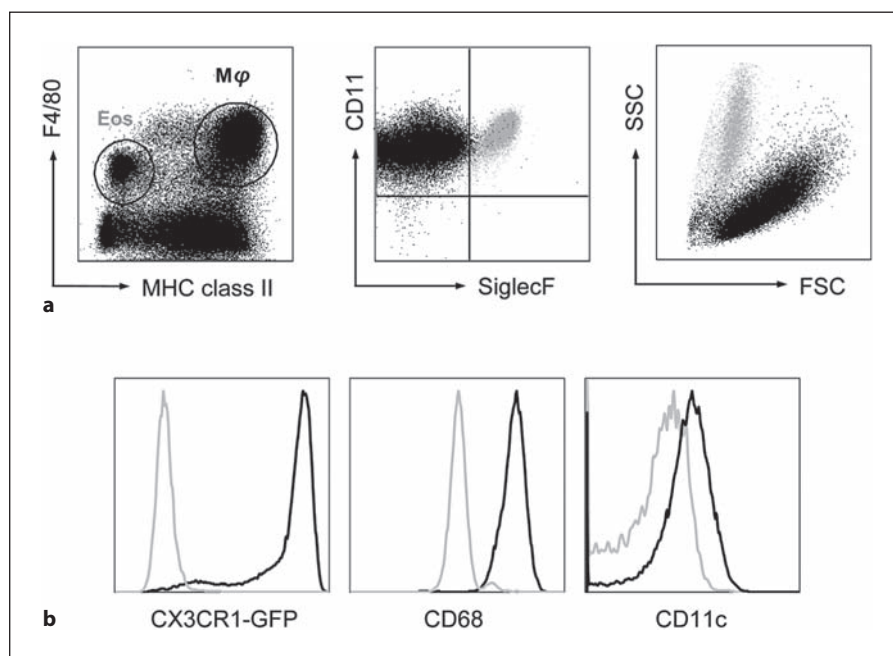
The intestine is exposed continuously to enormous amounts of antigens, some of which are potentially dangerous pathogens. As prominent effector cells of both innate and adaptive immune responses, mΦ would be expected to play important roles in protective immunity against such agents. Similar effector functions are engaged during inappropriate inflammatory responses against harmless local antigens, such as those seen in inflammatory bowel diseases (IBD) and activated mΦ are central to the pathogenesis of these disorders. Given that IBD is believed to be driven by the commensal microbiota that are present in large quantities in the normal large intestine and that mΦ are also always there, it is intriguing to consider why the intestine is not in a permanent state of inflammation. As we will discuss, this reflects a state of inertia in the resident mΦ in the normal intestine that allows these cells to play active and essential roles in maintaining epithelial integrity and mucosal homeosta-

sis. In this review, we will discuss the current knowledge of mΦ in the intestinal mucosa, highlight their phenotypic and functional heterogeneity and discuss how they may be able to play such opposing roles in healthy and inflamed gut.

How to Recognise a Mucosal MΦ

Mucosal mΦ show the morphological features of tissue resident mΦ, with a classical mononuclear shape and abundant, granular cytoplasm consistent with active phagocytic behaviour (fig. 1). In man they express the tissue mΦ marker macrosialin (CD68) and this is also present on most intestinal mΦ in mice (fig. 2) [3], together with the pan-mΦ markers F4/80, CD11b and the CSF-1 (M-CSF) receptor (CSF-1R; CD115) (table 1) [4–10]. Until very recently, studies of mouse mucosal mΦ have been complicated by the imprecise use of these and other markers to identify and purify them. Thus, relatively large numbers of CD11b+ F4/80+ eosinophils are present

Fig. 2. Phenotypic features of resident m Φ in healthy mouse colon. Two major populations of F4/80⁺ cells are present in populations of live CD45⁺ cells isolated by enzymatic digestion of mouse colon. The F4/80^{hi} class II MHC^{hi} cells are m Φ , as assessed by FSC, SSC and expression of CD68. Unlike resident m Φ in other tissues, they also express very high levels of CX3CR1 and significant amounts of CD11c. The cells that express lower levels of F4/80 are class II MHC⁻ and Siglec F⁺. They also have high SSC and are eosinophils.



in the normal mouse mucosa and it is necessary to discriminate these directly from m Φ on the basis of high SSC and selective expression of Siglec F by eosinophils (fig. 2). An even greater source of inaccuracy has been the assumption that the expression of class II MHC and CD11c allows dendritic cells (DC) to be distinguished from m Φ in mouse intestine. In our hands, mature resident m Φ from healthy mouse colon uniformly and constitutively express high levels of class II MHC (fig. 2) and human intestinal m Φ have also been reported to be class II MHC positive [10]. CD11c expression is also frequently used as a specific marker of murine DC, but well-defined intestinal m Φ can express substantial amounts of CD11c (table 1; fig. 2) [7, 9, 11–14].

These findings are compatible with the recent controversies over the relationship between different subsets of mononuclear phagocyte in mice, especially in non-lymphoid tissues, where the distinction between DC and m Φ is not as clear as in lymphoid organs [12, 15]. Importantly, recent studies have shown that many of the mucosal cells identified previously as DC on the basis of being CD11c⁺ class II MHC⁺ do not have the functions of classical mucosal DC [14]. Two major phenotypic populations of mucosal mononuclear phagocyte have now been proposed on the basis of expression of CD103 ($\alpha_E\beta_7$ integrin) and CX3CR1, the receptor for the chemokine fractalkine (FKN – CX3CL1) [for review, see 7, 16]. Although

both populations express CD11c and class II MHC, only the CD103⁺ CX3CR1⁻ cells possess the features of classical DC, presenting antigen to naïve T cells, migrating to MLN and imprinting gut homing markers and FoxP3 expression in naïve T cells via the production of retinoic acid (RA). Although some of these DC express the myeloid marker CD11b, they are F4/80⁻ CD68⁻. Conversely, CX3CR1⁺ cells in the mucosa are CD103⁻, but are F4/80⁺ CD11b⁺ CD68⁺ and do not migrate to MLN or prime naïve T cells, suggesting they are likely to be m Φ rather than DC. On these grounds, we would suggest that a consensus phenotype for m Φ in resting gut is F4/80⁺ CD11b⁺ CX3CR1⁺ CD103⁻ class II MHC⁺ Siglec F⁻ (fig. 2). It remains to be determined whether the same dichotomy applies in other species. Unfortunately, many of the studies discussed below did not use the multiparameter analyses that allow these subsets of mucosal mononuclear cells to be defined precisely and so must be interpreted with appropriate caution.

Functions of Resident Intestinal M Φ

In keeping with their activated appearance and expression of class II MHC, resident intestinal m Φ are highly phagocytic (fig. 1) and they express CD36, a receptor that allows phagocytic uptake of apoptotic cells [17].

Table 1. Phenotypic comparison of murine mΦ populations

Marker	Resident mucosal mΦ	Inflammatory mucosal mΦ	Monocyte	M1 mΦ	M2 mΦ	M2-like TAM
CD11b	+++	+++	+++	+++	+++	+++
F4/80	+++	++	+/-	+++	+++	++/+++
CD68	++	++	-	++	++	++
Class II MHC	+++	++/+	-	++	+	++
CD11c	++	++	-	++	-	+
CX3CR1	+++	++	-/+ ¹	-	-	++
CCR2	++	+++	++/- ¹	++	?	+
Ly6C	-	++ → -	++/- ¹	-	-	-
CD40	+/-	+/-	-	++	-	+
CD80, CD86	+/-	+/-	-	++	-	+
CD14	++	++	++	++	?	?
TLR2	++	++	+++	++	++	
TLR4	+/-	+	+++	++		
MannoseR	?	?	-	-	+	++
ScavengerR	?	?	-	-	+	++
Phagocytosis	+++	+++	+	+++	+/-	++
iNOS	-	++	+	+++	-	-
ROI	-	++	+	+++	-	-
TNF-α	±	+++	+	+++	-	+
IL-10	++	±	-	-	++	++
Arginase	-	-/+ ²	-	-	++	+
COX-2	++	?	-	++	+/-	++
IL-4R	-	-	-	-	++	-
IL-10R	+	-	-	-	-	++

Resident mucosal mΦ in resting mouse intestine are compared with the mΦ which infiltrate during intestinal inflammation, blood monocytes and the defined populations of polarised mΦ that have been described in mice: M1 ('classical' activated), M2 ('alternative' activated) and M2-like (tumour associated – TAM). ? = Not known.

¹ Two subsets of monocytes are described: 'resident' CX3CR1⁺ Ly6C^{lo} CCR2^{lo} vs. 'inflammatory' CX3CR1^{lo} Ly6C^{hi} CCR2^{hi}.

² A population of arginase-expressing mucosal mΦ is found in intestinal helminth infection.

They have also been reported to have strong bactericidal activity [10]. However, they do not express high levels of co-stimulatory molecules such as CD80, CD86 or CD40 [4, 10, 11, 18, 19]. Most significantly, unlike mΦ from other tissues, mucosal mΦ do not respond to stimuli such as TLR ligands by producing pro-inflammatory cytokines or chemokines such as IL-12, IL-23, TNF-α, IL-1, IL-6 or CXCL10 (IP-10) nor do they up-regulate co-stimulatory molecules or generate a significant respiratory burst and nitric oxide production under these conditions [5, 8, 10, 20–23]. Conversely, they produce the anti-inflammatory cytokine IL-10 constitutively or in response to TLR ligation [5, 13, 24–26]. While paradoxical at face value, this split personality is of considerable physiological importance, as ingestion and killing of microbes without initi-

ating overt inflammation would allow local mΦ to act as a firewall against any commensal bacteria that have crossed the overlying epithelial barrier. In this respect, it now appears that mΦ are the CX3CR1⁺ mononuclear phagocytes in the small intestinal lamina propria that may send processes through the epithelium to capture bacteria in the lumen [7, 14, 16]. Originally thought to be DC, these cells have never been shown to present the captured antigen to T cells. However, this luminal sampling appears to be required for the uptake and T cell recognition of non-pathogenic *Salmonella* organisms and soluble proteins [14, 27, 28]. The role of these CX3CR1⁺ mΦ may therefore be to pass the material on to DC for transport to the MLN. Alternatively, mΦ may degrade their captured contents in a non-inflammatory manner as dis-

cussed above, or may migrate into the lumen itself in a Myd88-dependent manner, providing a layer of cellular innate defence [29].

MΦ Handling of Intestinal Bacteria and Disease Susceptibility

The ability of intestinal mΦ to deal with local commensal bacteria in an appropriate manner is essential for homeostasis, as genes that interfere with these processes are associated with susceptibility to IBD in man [30]. Most notable of these is the intracellular pattern recognition receptor NOD-2 present in mΦ that recognises muramyl dipeptide in peptidoglycan from bacterial cell walls. Around 30% of patients with small intestinal Crohn's disease have a non-functional polymorphism in the NOD-2 gene, the strongest genetic linkage yet discovered in human IBD [31, 32]. Knock-out of NOD-2 in experimental animals has rather inconsistent effects, but overall, it is associated with a failure of mΦ to generate appropriate innate responses to bacteria [31]. Autophagy is a further important mechanism in the control of innate immune responses to intracellular bacteria and viruses, and polymorphism in the autophagy-associated gene Atg16L1 has also been associated with susceptibility to Crohn's disease [33, 34]. Atg16L1 is expressed highly by intestinal epithelial cells and is also functional in mΦ [35, 36]. The immunity-related GTPase family M (IRGM) protein is also involved in autophagy and innate defence to intracellular organisms and genetic polymorphism in IRGM is linked to Crohn's disease [37, 38]. Together these findings support the view that dysregulated mΦ responses to intestinal bacteria are important factors in human IBD.

Anti-Inflammatory Properties of Resident Intestinal MΦ

Resident intestinal mΦ do not just contribute to intestinal homeostasis by acting as a waste disposal unit for local bacteria, but also actively regulate epithelial integrity. As a result, depletion of resident mΦ increases susceptibility of mice to experimental colitis and acute intestinal graft versus host disease [9, 39, 40]. Among the mechanisms that may account for this trophic effect is cyclo-oxygenase 2-dependent production of prostaglandin E2 by mΦ located in the stem cell niche of the epithelium [41]. Whether this is the only mediator that enables

mucosal mΦ to regulate epithelial cell renewal and whether mΦ act directly on epithelial stem cells or via other cell types such as mesenchymal cells remains to be established. Interestingly, cyclo-oxygenase 2 may also contribute to one further active function of intestinal mΦ, namely their production of the anti-inflammatory cytokine IL-10 [42]. Expression of the transcription factor peroxisome proliferator-activated receptor- γ (PPAR- γ) by mucosal mΦ is a further mechanism by which resident mΦ can prevent local inflammation, via its ability to suppress pro-inflammatory gene expression [43] and by restricting CCR2-dependent entry of pro-inflammatory monocytes into the intestine [44].

MΦ and Specific Tolerance to Intestinal Antigens – An Unexpected Alliance?

Recently, an additional and somewhat surprising physiological role for intestinal mΦ has emerged, namely in regulating antigen-specific tolerance. This was first suggested by evidence that F4/80 KO mice did not develop tolerance or antigen-specific CD8⁺ regulatory T cells (Treg) normally after feeding soluble antigen, but the cellular and anatomical processes underlying this defect were not determined [45]. CD11b KO mice also have a defect in oral tolerance, but this could reflect the absence of either mΦ or CD11b⁺ DC [46]. More recent studies have shown that mΦ-derived IL-10 is important for maintaining local regulatory T cell differentiation in the intestine and that this is needed to prevent inflammatory reactions against commensal bacteria and food proteins [5, 13, 25]. As these mΦ cannot migrate to meet naïve CD4⁺ T cells in MLN in the resting condition, their role may be to maintain the survival of Treg in the mucosa. This idea has been confirmed by recent elegant studies which show that IL-10-producing CX3CR1⁺ mΦ in the small intestinal mucosa are needed for the induction of local and systemic tolerance after feeding protein antigens [25]. In these experiments, tolerance was associated with FoxP3⁺ Treg whose differentiation was initiated in the MLN by antigen loaded, RA producing CD103⁺ DC. Having acquired gut homing markers, the Treg exited the MLN and migrated to the mucosal lamina propria where further clonal expansion and terminal differentiation occurred under control of IL-10-producing CX3CR1⁺ mΦ. This result is reminiscent of other recent work which suggests that effector T cell differentiation in tissues such as the skin may require two separate 'hits' from antigen and APC, one in the draining LN and the second via DC in

the tissue itself [47]. Resident human mucosal m Φ have been found to produce CCL20 (MIP3 α) whose ligand CCR6 is expressed on FoxP3+ Treg and this may encourage local interactions between m Φ and Treg in the healthy gut [48]. Interestingly both the generation of Treg and tolerance were absent in CX3CR1 KO mice, not because CX3CR1+ m Φ failed to accumulate in the mucosa, but because they could not produce IL-10 once there [25]. Together these findings support a critical role for mucosal m Φ in shaping local and systemic immune responses to intestinal antigens.

Intestinal M Φ and Their Relatives

A number of functional m Φ subsets have been described in mice, including 'classical' (M1) and 'alternatively' activated (M2) m Φ which contribute to Th1- and Th2-mediated forms of immune response, respectively, by producing distinctive patterns of mediators (table 1). Within the M2 population there is also a group of m Φ with immunoregulatory and anti-inflammatory properties that are very similar to those of resident mucosal m Φ . These 'M2-like' m Φ express CD163 and CD206 lectin receptors, and produce IL-10 as well as tissue remodelling factors such as VEGF, metalloproteinases and activin. In parallel, they cannot produce pro-inflammatory mediators in response to stimulation [49, 50]. Often found infiltrating tumours and during tissue repair, these 'M2-like' cells are also usually class II MHC+ and phagocytic, underlining their similarity to mucosal m Φ [10, 50]. Thus, m Φ in the healthy intestine may be part of a functional subset involved in the physiological processes of tissue remodelling and prevention of inflammation against commensal microbes.

Mechanisms of Inertia in Mucosal M Φ

The molecular reasons underlying the functional inertia of mucosal m Φ remain to be established with certainty, although a number of possibilities have been proposed. Many studies have focussed specifically on their inability to respond to TLR ligands and several reports suggested that resident intestinal m Φ in both humans and mice failed to express TLR or associated receptors such as CD14 that are normally present at high levels on monocytes and m Φ [4, 8, 10, 21, 51–53]. However, there is now considerable evidence to suggest that these cells express many, if not all TLR both at the mRNA and pro-

tein level [10, 54]. In our hands, murine resident colon m Φ express high levels of surface TLR2 protein, yet are unresponsive to stimulation via TLR2 ligands [unpubl. observations]. The position with CD14 is less clear, as most mouse intestinal m Φ express high levels of this molecule, whereas human m Φ may not [10, 14]. What is clear is that there is a functional block in signalling through the TLR receptor pathways in intestinal m Φ and they fail to activate and translocate NF- κ B p65 to the nucleus in response to appropriate stimuli [54]. Several mechanisms have been proposed to explain these defects, including absence of a number of downstream effectors and adaptors in the TLR signalling cascade, such as MyD88, IL-1 receptor-associated kinase (IRAK), IRF2, TRAF-6 and TRIF-associated adaptors [54–56]. Other abnormalities that have been reported include expression of the IRAK-1 inhibitor, IRAK-M [57], and failure to express gp96. This estrogen receptor-associated chaperone is required for folding and shuttling TLR to the cell surface or endosomes [58, 59]. Over-expression of the E3 ubiquitin-protein ligase, Triad3A, which mediates degradation of some TLR has also been reported [60], as has induction of SOCS1 inhibitor of TLR signalling [54].

It is important to recognise that the functional hyporesponsiveness of intestinal m Φ extends beyond TLR ligation and applies to all stimuli that have been explored. These include ligands for pattern recognition receptors such as NOD-1/NOD-2 or C-type lectins, intact bacteria or apoptotic debris, IFN- γ and even PMA [10, 19, 24]. In addition, human intestinal m Φ lack expression of other stimulatory receptors such as Fc γ RI and Fc γ RIII, the CR3 and CR4 complement receptors [17], Fc α R [52] as well as the triggering receptor expressed on myeloid cells-1 (TREM-1) [26, 61]. The extensive nature of these defects makes the inertia of mucosal m Φ quite distinct from the well-known phenomenon of 'endotoxin tolerance' in which exposure to a single TLR ligand suppresses subsequent responses to the same ligand. Rather, there is a profound global extinction of stimulatory potential in mucosal m Φ that ensures they do not respond inappropriately to any of the potentially inflammatory constituents of their environment.

Does Homeostatic Inertia of Intestinal M Φ Require TLR Ligation?

Despite the lack of overt inflammation in the intestine and the general unresponsiveness of mucosal m Φ to TLR stimulation, there is considerable evidence that their state

of inertia may require prior TLR ligation. TLR signalling in both epithelial cells and m Φ is essential for protecting the intestinal epithelium against mechanical and toxic insults [62–64]. In addition, our recent findings suggest that there may be a low level of constitutive production of TNF- α by resident colonic m Φ [unpubl. observations]. It is possible that this does not go on to cause pathology because it is normally balanced by the concomitant constitutive production of IL-10 or by the effects of TGF- β discussed above. Work on tumour-infiltrating m Φ has shown that their regulatory phenotype may reflect NF- κ B-mediated activation of anti-inflammatory pathways due to TLR ligation in the context of special microenvironmental factors such as extracellular matrix or hypoxia [65]. However, very recent studies suggest that TLR and local bacteria may not be essential for the inertia in mucosal m Φ , as it can be seen in the sterile gut during foetal development, secondary to local production of TGF- β [66]. Thus, a number of mechanisms may ensure that any pro-inflammatory responses are extinguished sufficiently rapidly to avoid damage in the normal intestine.

Local Conditioning of M Φ Function in the Intestine

As the most likely origin of mucosal m Φ is blood monocytes (see below) and these are fully responsive to stimulation, it is probable that factors in the intestinal environment may be responsible for ‘conditioning’ intestinal m Φ to become unresponsive after their arrival in the gut. There are many possible candidates for this activity, but IL-10 and TGF- β stand out. In conjunction, these cytokines can reproduce TLR or NOD-2 unresponsiveness and they down-regulate TREM-1 and CD89 expression in human blood monocytes [61, 67, 68]. As we have noted, IL-10 is produced constitutively by mucosal m Φ themselves and there are also large numbers of IL-10-producing T lymphocytes in the normal intestinal mucosa [69]. IL-10 KO mice develop spontaneous IBD, as do mice in which IL-10 signalling in m Φ has been abolished by targeted KO of Stat3 in myeloid cells [70]. Inhibiting autocrine/paracrine production of IL-10 by mucosal m Φ reverses their TLR unresponsiveness *in vitro* [5] and the role of IL-10 is likely to reflect its ability to prevent NF- κ B activation in m Φ . This may occur via inhibition of I κ B kinase that normally releases NF- κ B from its inhibitory partners, or by induction of mechanisms that prevent NF- κ B binding to DNA such as the inhibitory I κ B family members, I κ BNS and Bcl-3 [19, 71, 72]. Clearly, IL-10 is an important physiological regulator of intestinal m Φ function.

TGF- β is a ubiquitous cytokine with many immunomodulatory and anti-inflammatory properties that are important in the intestine, including the maintenance and function of regulatory T cells and the switching of IgA production in naïve B lymphocytes [73, 74]. TGF- β is produced abundantly by many different cells in the mucosa, including haematopoietic cells, epithelial cells and mesenchymal stromal cells [10], and it plays important roles in regulating epithelial cell differentiation and renewal [75]. TGF- β itself is a potent inducer of M2-like regulatory m Φ differentiation [50] and FoxP3+ Treg can also do this in a partially TGF- β -dependent manner [76]. As we have discussed, it is now clear that m Φ interact closely with Treg in the mucosa [25], and recent studies have shown that resident human mucosal m Φ express high levels of TGF- β receptors. In parallel they have constitutively active TGF- β signalling via the Smad4 transcription factor [54]. TGF- β itself induces the expression of inhibitory I κ B α in m Φ and also promotes ubiquitinylation and degradation of MyD88 [76]. The inability of mucosal m Φ to activate and translocate NF- κ B after TLR stimulation treatment can be reproduced in blood monocytes by treatment with TGF- β derived from intestinal stromal cells [54]. In addition, TGF- β from adult intestine prevents foetal intestinal m Φ from responding to TLR ligation [66]. Altogether, these findings indicate that TGF- β derived from a number of sources is likely to be key to determining m Φ behaviour in the intestine and may be at the centre of a crucial two-way homeostatic interaction between m Φ and Treg. Importantly, mucosa from patients with Crohn’s disease shows an intrinsic resistance to TGF- β receptor signalling, indicating how dysregulation of this pathway may be of clinicopathological significance [77].

A variety of other factors could be involved in conditioning local m Φ inertia. These include mediators that have been shown to have similar effects on intestinal DC, such as RA and thymic stromal lymphopoietin [78], or agents that are produced in high quantities in the gut and that have been reported to drive anti-inflammatory m Φ in other sites, such as vasoactive intestinal peptide [79]. As noted above, CX3CL1 has been implicated in inducing IL-10 production by mucosal CX3CR1+ m Φ [25] and this chemokine is produced by intestinal epithelial cells in large quantities [80]. Similar conditioning effects of CX3CL1 have been shown for m Φ from other tissues such as the brain [81], but contradictory reports have also suggested that CX3CL1 may activate m Φ [3] and this issue needs to be examined more carefully.

Inhibitory Receptors and MΦ Inertia

One further possibility we have considered to explain the unresponsiveness of mucosal mΦ is that there could be constitutive ligation of potentially inhibitory receptors. A candidate we considered was CD200 receptor 1 (CD200R1), an Ig superfamily molecule expressed at high levels on myeloid cells that recognises the ubiquitously expressed ligand CD200 and induces negative signalling by as yet unknown mechanisms [82]. Knock-out of CD200 or CD200R1 leads to mΦ hyperactivity and autoimmune disease in vivo [83, 84], and recent studies have shown that deleting or inhibiting the CD200-CD200R1 interaction renders mice more susceptible to lung inflammation during infection with influenza virus [85]. This correlates with CD200R1 expression by alveolar mΦ, a feature which is also characteristic of mΦ from resting colon (fig. 3). Together with the fact that CD200R1 can be induced by IL-10 and TGF-β [85], this led us to hypothesise that lack of CD200R1 might also predispose to intestinal inflammation. However, preliminary results indicate that CD200R1 KO mice have normal intestinal mΦ populations, do not develop spontaneous IBD and are not more susceptible to colitis induced by oral administration of DSS (fig. 3) [unpubl. observations]. Thus, CD200R1 does not appear to play an essential role in regulating intestinal mΦ behaviour.

Finally, the intestine is rich in endogenous and exogenous lipid ligands for PPAR-γ, and targeted deletion of PPAR-γ in myeloid cells under the lysozyme promoter leads to spontaneous activation of mucosal mΦ as well as increased susceptibility to experimental colitis. In turn, these pathological features can be prevented in intact mice by administration of exogenous PPAR-γ ligands [44, 86]. Thus, there may be several levels of local control that normally ensure down-regulation of intestinal mΦ function.

Intestinal MΦ in Inflammation

The phenotype and behaviour of mucosal mΦ change dramatically during protective immune responses or in inflammation. Under these circumstances, there is an intense infiltration of mΦ that express higher levels of TLR, CD14, co-stimulatory molecules, TREM-1 and other pro-inflammatory receptors than their resident counterparts [10, 18, 53, 87]. These mΦ also produce large quantities of mediators such as TNF-α, IL-1, IL-6, nitric oxide, reactive oxygen intermediates, cathepsins and metallopro-

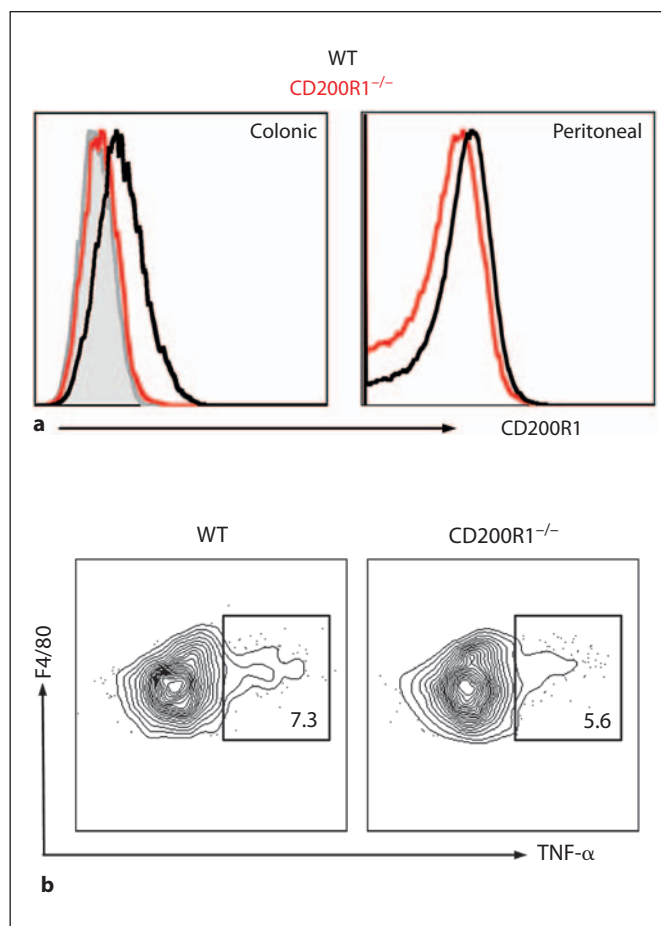


Fig. 3. CD200R1 and intestinal mΦ function. Resident colonic mΦ express CD200R1 (a), but lack of CD200R1 does not affect the production of TNF-α by purified colonic F4/80^{hi} class II MHC^{hi} mΦ in response to the TLR2 ligand bacterial lipopeptide (b).

teases [20, 88–91]. As a result, inflammatory mΦ play crucial roles in the pathogenesis of Crohn's disease and other forms of IBD, as well as in protection against intracellular infections such as salmonellosis, cryptosporidiosis and toxoplasmosis [10, 92–94]. The major role of mucosal mΦ in protective immunity is likely to be mainly as non-specific effector cells, rather than in initiating the adaptive immune responses, as the entry of and immune responses to fully pathogenic *Salmonella* that normally enter via Peyer's patches do not require CX3CR1⁺ mucosal mΦ to capture luminal bacteria [95].

An important but unresolved issue is whether the changes in mΦ behaviour that occur in inflammation reflect alterations in the normally unresponsive resident population, or if there is recruitment of new, fully respon-

sive cells (fig. 4). Currently, most evidence favours the latter idea, but it is unclear if these newly arrived m Φ belong to a distinct lineage from those responsible for maintaining homeostasis in the healthy intestine.

Ontogeny and Recruitment of Intestinal M Φ in Inflammation

The lack of precise markers for identifying mucosal m Φ means that many studies which have attempted to examine their origins were focussed mostly on defining subsets of DC and ignored the possibility that m Φ might also have been present.

Despite this proviso, it seems clear that inflammatory m Φ in mouse intestine are derived from a newly recruited and recently divided population of Ly6C^{hi} blood monocytes (fig. 4). This would be consistent with work in other tissues [49, 96] and when transferred into mice lacking all m Φ and DC, Ly6C^{hi} monocytes give rise to a population of CD11b⁺ CD11c⁺ CX3CR1⁺ E-cadherin⁺ class II MHC⁺ m Φ in the mucosa. These can produce copious amounts of TNF- α and other inflammatory mediators [7, 27, 28, 97].

A similar recruitment of cytokine producing monocyte-derived CD14⁺ m Φ is seen in the lesions of active Crohn's disease in man [21, 24, 51, 61, 88] and so there is considerable interest in exploiting the mechanisms responsible as targets for therapy. The Ly6C^{hi} monocytes that give rise to inflammatory m Φ in the mouse intestine are CCR2⁺ and their accumulation is dependent on this chemokine receptor in the same way as it is in other inflamed sites [8, 49]. In parallel, increased levels of CCR2 ligands can be found in inflamed mucosa and experimental colitis in mice is abolished in CCR2 KO mice [8, 10]. Other factors that may contribute to the recruitment of inflammatory m Φ include CCR5 [98, 99] and enhanced expression of the adhesion molecules P-selectin glycoprotein ligand-1, VCAM-1, CD31 and ICAM-1 on endothelial cells in the inflamed mucosa [100, 101]. The progeny of Ly6C^{hi} monocytes in T cell-dependent colitis in mice also express CCR6 [97], whose ligand CCL20 is present in the intestine, but it is not known if CCR6 is required for the recruitment of inflammatory m Φ to the intestine. It has also been reported that mice lacking the CX3CR1 chemokine receptor have a defect in the recruitment of m Φ to the inflamed colon and spleen, as well as being resistant to experimental colitis [3, 49, 102]. Thus, several different factors may play redundant and/or overlapping roles in recruiting monocytes to the inflamed gut.

Ontogeny and Recruitment of Resident Intestinal M Φ

The origins of the resident population of m Φ found in healthy intestine have been less easy to identify (fig. 4), but their presence is dependent on the myeloid growth factors CSF-1 and GM-CSF, and independent of the DC-specific flt3 tyrosine kinase receptor [7, 9, 27]. In mice, it is believed that a discrete population of CX3CR1⁺ Ly6C[–] monocyte gives rise to tissue resident m Φ [49]. However, the entry of such monocytes into the intestine has never been shown directly. Indeed the only study to have examined this could not find any progeny of transferred CX3CR1⁺ Ly6C[–] monocytes in the intestine, even in mice depleted of all m Φ and DC [28]. As kinetic studies indicate that resident CX3CR1⁺ m Φ turn over very slowly in vivo [14, 16], the entry of their precursors into the normal gut may be a relatively rare event and difficult to demonstrate experimentally. Somewhat surprisingly, inflammatory Ly6C^{hi} monocytes have been shown to repopulate intestinal m Φ in the absence of overt inflammation, although this can only be seen when the resident populations have been ablated for example by irradiation or diphtheria toxin-mediated depletion of CD11c⁺ myeloid cells [7, 27]. One interpretation of this is that the antigenic challenge faced by the normal intestine may make it behave like an 'inflamed' tissue in terms of its m Φ renewal. Certainly this would be consistent with the fact that all resident m Φ in mouse mucosa express CCR2 [26, unpubl. observations], a receptor that is normally associated specifically with the inflammatory Ly6C^{hi} subset of monocyte. Although it is difficult to exclude the possibility that the conditioning regimes necessary to demonstrate entry of m Φ into the otherwise healthy intestine induce mild inflammation, the entry of IL-10-producing m Φ into normal mouse intestine is dependent on the chemokine CCL2, one of the ligands for CCR2 [103]. Therefore, the monocytes that give rise to m Φ in resting intestine may be distinct from those that replenish other tissues.

Given these issues, it is not surprising that the factors responsible for recruiting resident m Φ to the healthy gut are also unclear. Although it has been shown that TGF- β and IL-8 produced by intestinal stromal cells may act as chemotactic factors for resting monocytes [104], the role of these factors in recruiting resident intestinal m Φ in vivo has yet to be proved. Despite the high levels of expression of CX3CR1 on resident mucosal m Φ and its possible role in recruiting inflammatory m Φ , the CX3CR1-CX3CL1 axis does not seem to play an essential role in

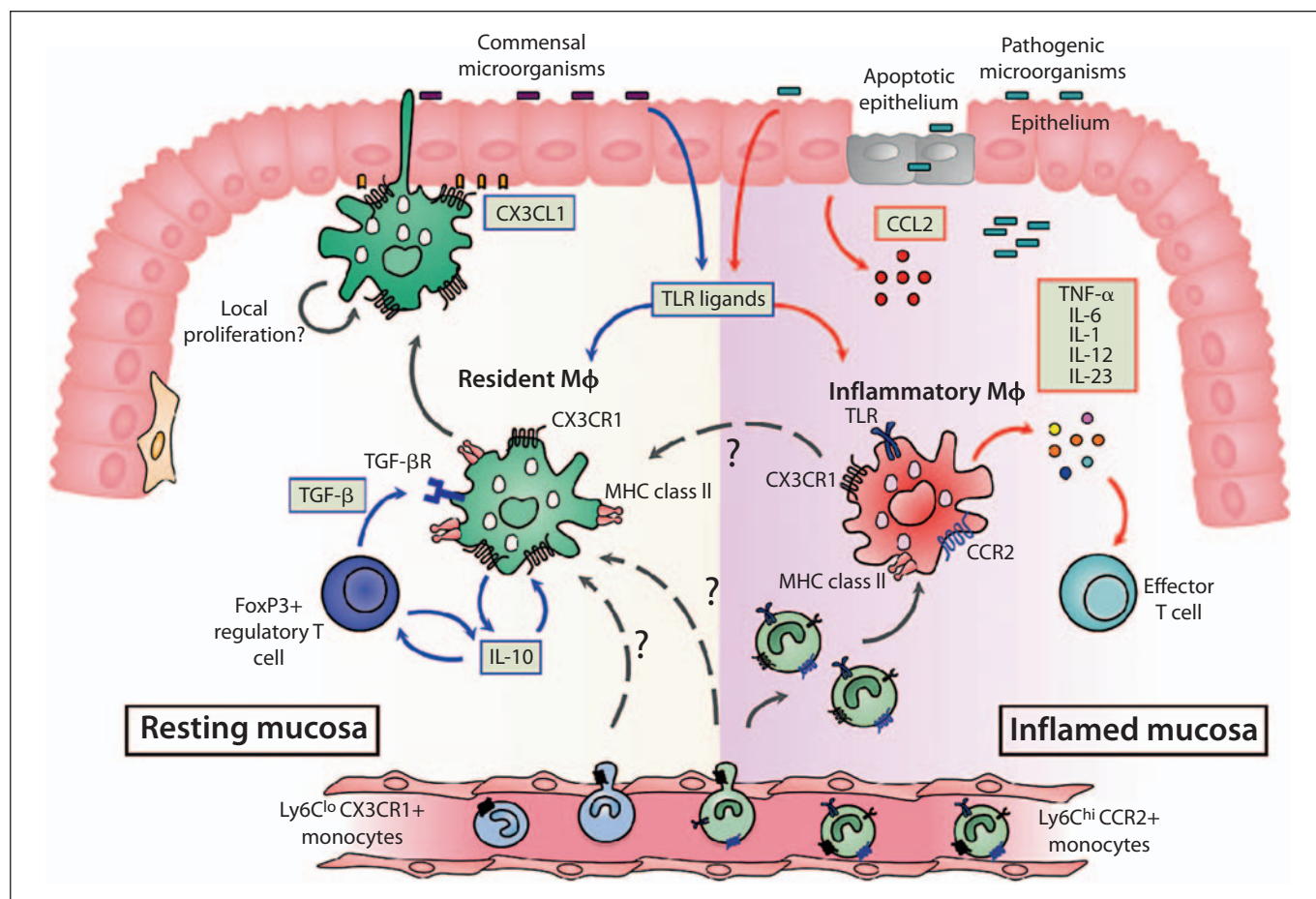


Fig. 4. Origin and diversity of intestinal mΦ in healthy and inflamed intestine. In resting intestine, the majority of intestinal mΦ express high levels of CX3CR1, the receptor for the transmembrane chemokine fractalkine (CX3CL1) expressed by intestinal epithelial cells. These mΦ can ingest and kill commensal bacteria, perhaps by extending processes across the epithelial barrier into the lumen. They produce IL-10 constitutively, but are unable to produce pro-inflammatory mediators in response to TLR or other stimuli. This means that resident intestinal mΦ cannot assist effector T cell activity, but instead, their production of IL-10 is needed to maintain the survival of local FoxP3⁺ Treg. In turn, the Treg produce TGF-β which, together with IL-10 and other local factors, maintain resident mΦ in their state of partial inertia. This may also require TLR signalling from the commensal microbiota. The resident mΦ are long-lived, and may be

replenished directly from the bloodstream either by resident CX3CR1⁺ Ly6C^{lo} monocytes or by inflammatory CX3CR1^{lo} Ly6C^{hi} monocytes. There may also be local turnover of resident mΦ, or differentiation of the small numbers of CX3CR1^{int} inflammatory mΦ present in normal mucosa (see text for details). After breach of the epithelial barrier, or pathogenic invasion, the mucosa is infiltrated by large numbers of inflammatory mΦ which express lower levels of CX3CR1. These CX3CR1^{int} mΦ are derived from recently divided inflammatory CX3CR1^{lo} Ly6C^{hi} CCR2⁺ monocytes in the bloodstream, recruited in response to increased levels of CCR2 ligands. The CX3CR1^{int} mΦ produce large amounts of pro-inflammatory mediators that drive local inflammation and promote the function of effector T cells. Some inflammatory CX3CR1^{int} mΦ may eventually differentiate into resident CX3CR1^{hi} mΦ to assist resolution of damage.

mΦ recruitment to the healthy intestine, as CX3CR1 KO mice have normal populations of resident mΦ [28, 105, unpubl. observations]. However, as noted above, CCR2 may play a counter-intuitive role in recruiting both resident and inflammatory mΦ in contrast to its pro-inflammatory role in other tissues.

Self-Renewal of Intestinal MΦ as a Local Mechanism of Replenishment

One possible explanation for the difficulties in identifying the precursors of resident intestinal mΦ is that they could be replenished by local self-renewal in situ

(fig. 4). This is known to occur with some other mononuclear cell populations, including microglia in the central nervous system and Kupffer cells in the liver, as well as for the DC-related Langerhans cells in the epidermis of the skin [49, 106–108]. In these cases, the resident population is established before, or just after birth, by a myeloid precursor which subsequently self-renews. There may also be a contribution from blood-derived monocytes throughout normal adult life and, during inflammation, there is an intense recruitment of inflammatory monocytes that dilutes out the resident m Φ population, as occurs in inflamed intestine [26]. The possibility that self-renewing m Φ may exist in the gut has not been addressed directly, but adoptively transferred Ly6C^{hi} monocytes appeared to undergo clonal expansion in villus mucosa in m Φ /DC-depleted recipients [28]. In addition, mucosal m Φ are absent in CSF-1 receptor KO mice and after antibody-mediated neutralisation of CSF-1R [9, 27], despite the fact that blood monocytes can repopulate other tissue m Φ in CSF-1R KO mice [108]. Thus, the need for CSF-1 may be in maturation of local precursors [9], and if these findings can be confirmed in physiological conditions, they would suggest that self-renewal of m Φ might be an important process in the healthy intestine.

CX3CR1 Expression as a Marker of Resident and Inflammatory M Φ in the Intestine

One of the reasons so little is known about the origins and recruitment of resident and inflammatory m Φ in the intestine has been the lack of precise phenotypic markers with which to distinguish these cells. Very recent work may provide some novel insights into this, by showing that two distinct subsets of inflammatory and resident m Φ can be identified in the intestine on the basis of their level of CX3CR1 expression [26, unpubl. observations]. In the resting state, the majority of mucosal m Φ express levels of CX3CR1 that are higher than any other myeloid population (fig. 2), and this resident subset expresses mRNA for anti-inflammatory factors such as IL-10, Homx1 and the mannose receptor; it is also unresponsive to stimulation *in vitro* [26]. The ‘inert’ status of these CX3CR1^{hi} m Φ changes little during inflammation, when they are diluted out by a TLR-responsive population which expresses lower levels of CX3CR1, as well as producing TNF- α and other inflammatory mediators such as IL-6 and iNOS [26]. Both subsets are CD11b^{hi} F4/80+ class II MHC+ and are heterogeneous for CD11c expres-

sion, meaning they cannot be distinguished on the basis of these conventional markers. Interestingly, a small number of these CX3CR1^{int} m Φ is also found in the normal intestine and it is tempting to speculate that it is these active cells which account for the homeostatic functions of m Φ in the healthy gut.

Conclusions

M Φ are a fundamental component of the innate immune system in the healthy and inflamed intestine, where they play essential roles in homeostasis and disease. In normal gut, they are held in a state of partial inertia, but this changes rapidly during inflammation, by as yet unknown mechanisms. Although mucosal m Φ are now clearly heterogeneous in nature, it is not known whether inflammation reflects plasticity within individual subsets or is due to changes in the balance of their recruitment. Importantly, although inflammatory m Φ seem to be derived from Ly6C^{hi} monocytes, the precursor of resident intestinal m Φ has not been identified directly. Whether this is the CX3CR1+ ‘resident’ monocyte, whether they are generated from local conditioning of initially inflammatory m Φ or whether they are maintained by local turnover and renewal, as can occur with some m Φ in other tissues are important questions for future work. These crucial studies will inform the specificity and safety of new therapeutic strategies aimed at targeting the recruitment and/or activation of one or other of the m Φ subsets.

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